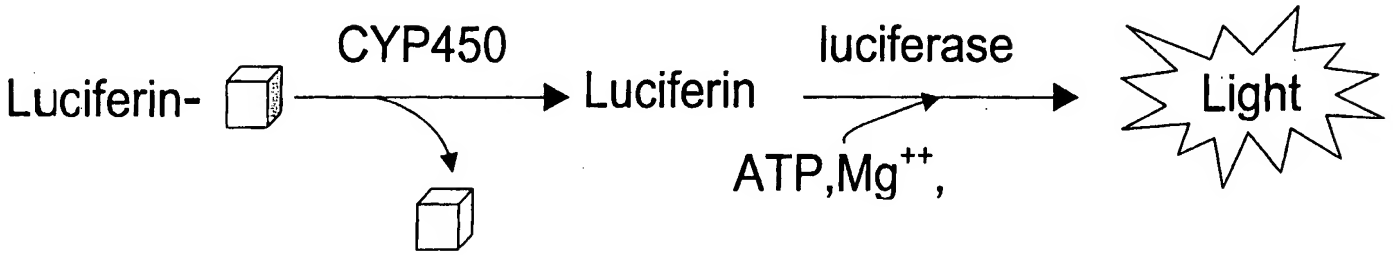
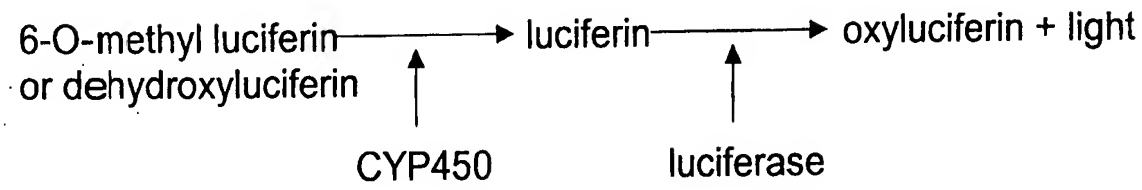
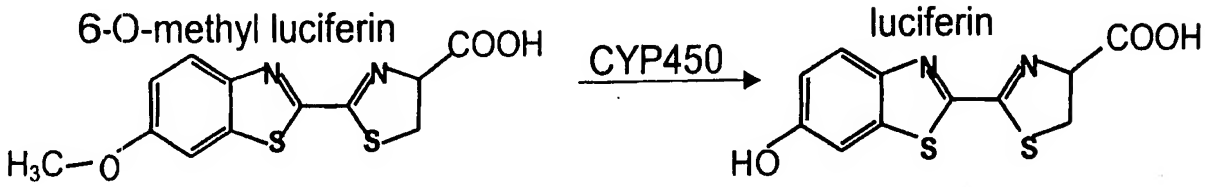
Figure 1



Examples





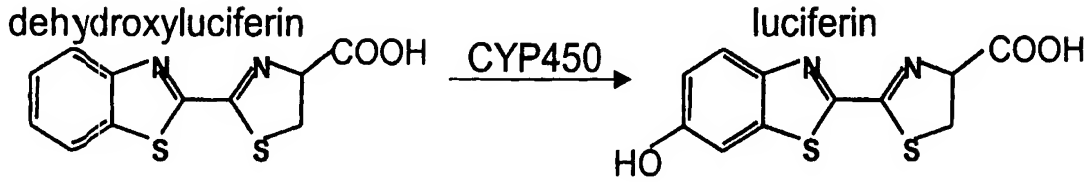
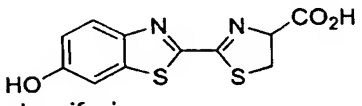
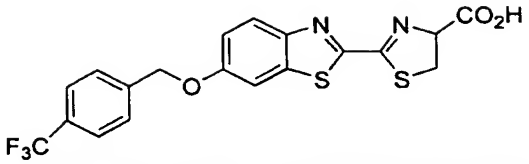
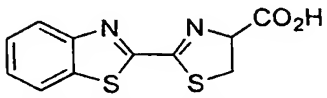


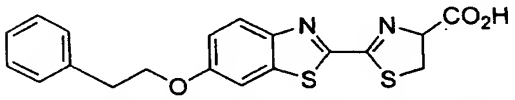
Figure 2

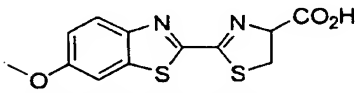
D-luciferin and D-luciferin derivatives.

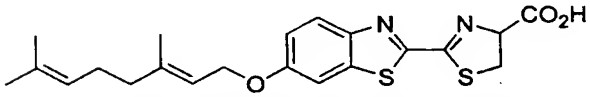

Luciferin

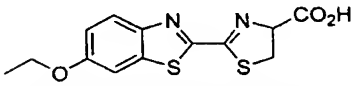

Luciferin 6' 4-trifluoromethylbenzyl ether (LucTFMBE)

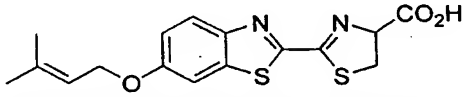

Dehydroxyluciferin (Hluc)

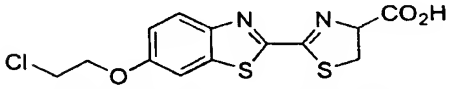

Luciferin 6' phenylethyl ether (LucPEE)

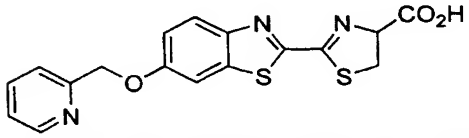

Luciferin 6' methyl ether (LucME)

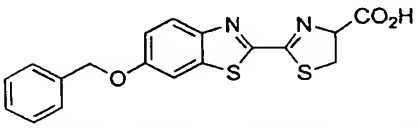

Luciferin 6' geranyl ether (LucGE)

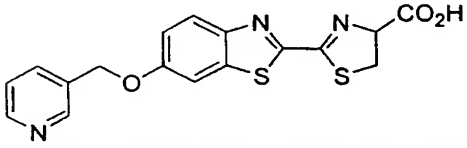

Luciferin 6' ethyl ether

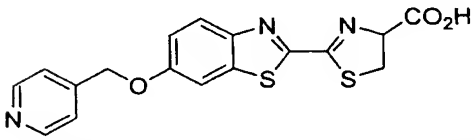

Luciferin 6' prenyl ether (LucPE)


Luciferin 6' 2-chloroethyl ether (LucCEE)


Luciferin 6' 2-picolinyl ether (Luc2PE)


Luciferin 6' benzyl ether (LucBE)


Luciferin 6' 3-picolinyl ether (Luc3PE)


Luciferin 6' 4-picolinyl ether (Luc4PE)

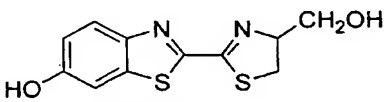
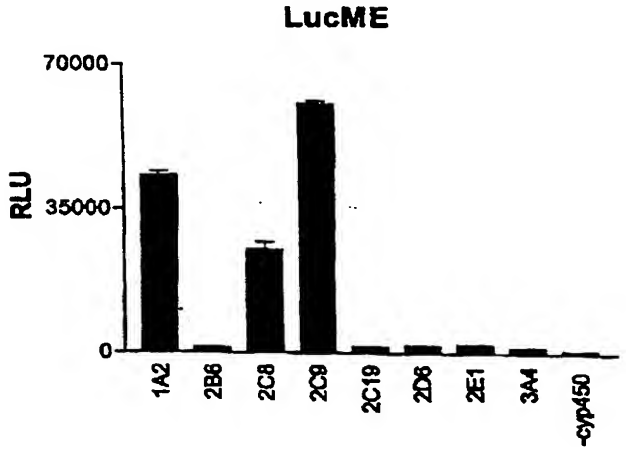

Luciferol

Figure 3

Two-step luminescent cyp450 reactions using D-luciferin derivatives

A



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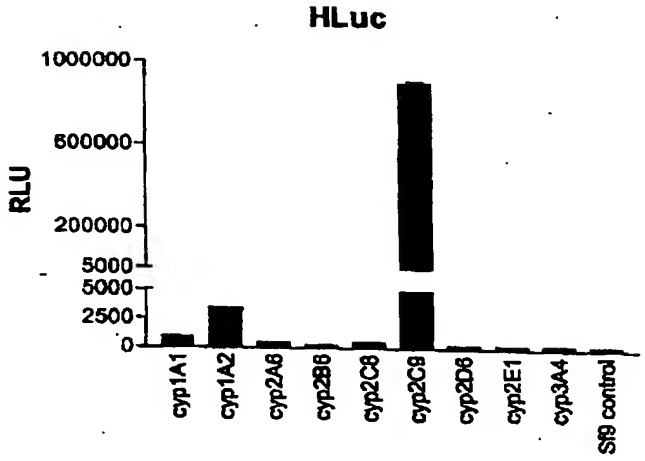
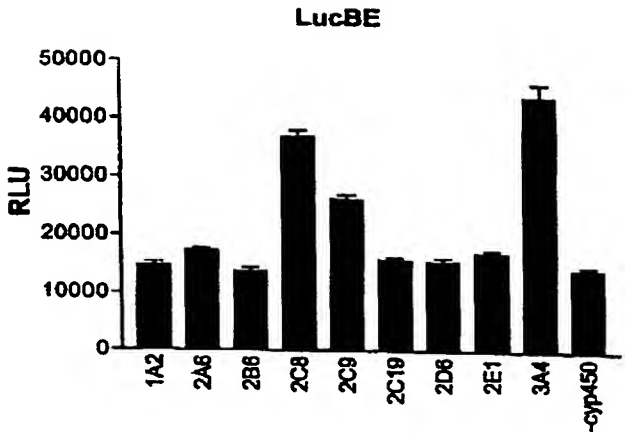
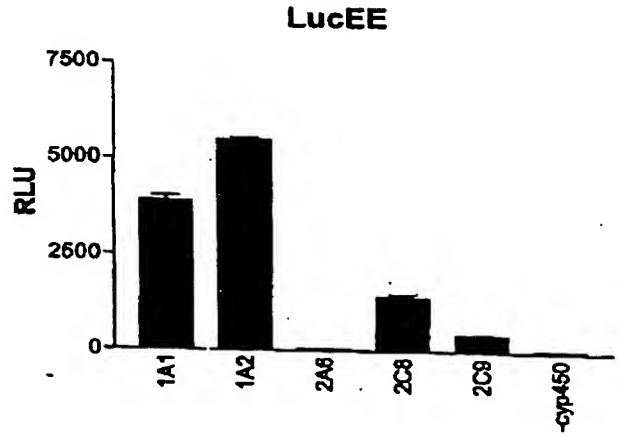


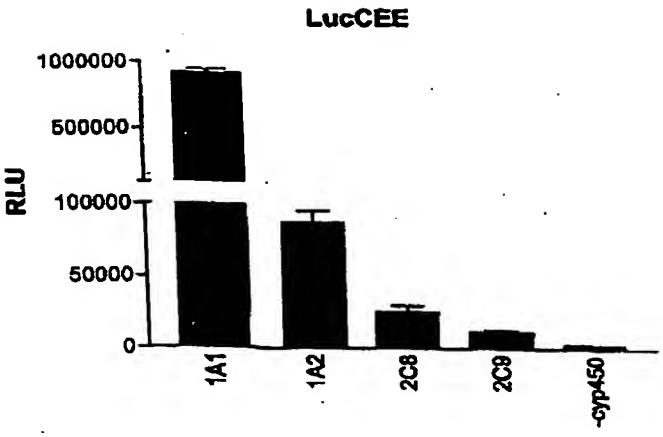
C


LucBE
RLU
50000
40000
30000
20000
10000
0
1A2
2A6
2B6
2C8
2C9
2C19
2D6
2E1
3A4
-cyp450

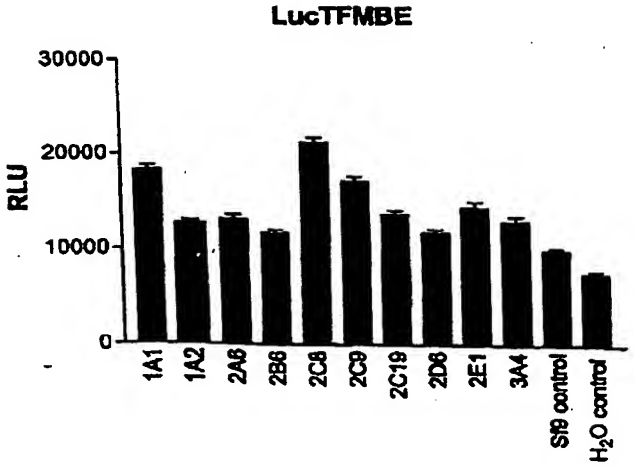
D


LucEE
RLU
7500
5000
2500
0
1A1
1A2
2A6
2C8
2C9
-cyp450

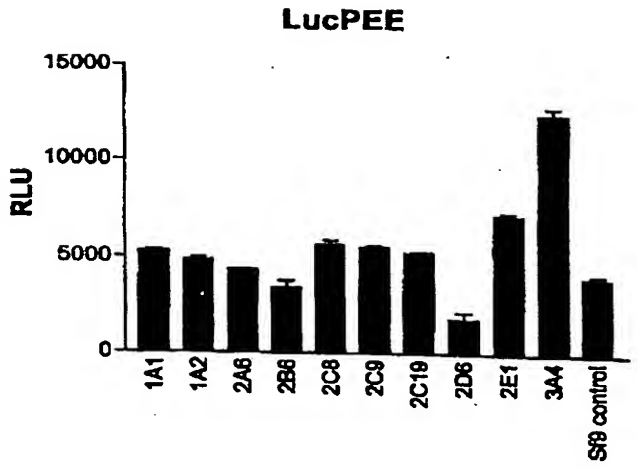
E


LucCEE
1000000
500000
100000
50000
0
RLU
1A1
1A2
2C8
2C9
-cyp450

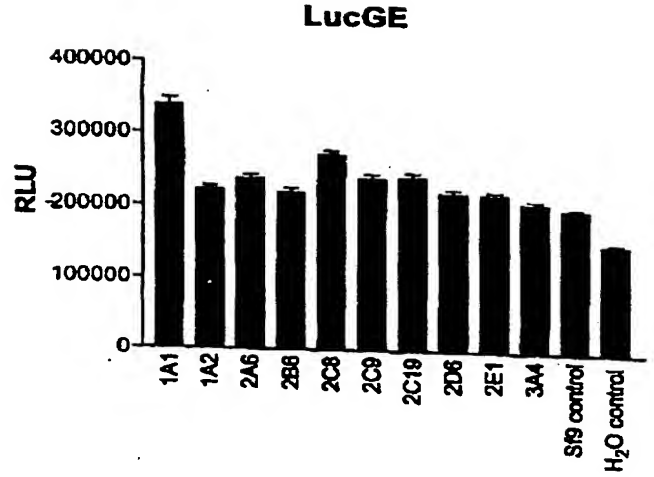
F


LucTFMBE
30000
20000
10000
0
RLU
1A1
1A2
2A6
2B6
2C8
2C9
2C19
2D6
2E1
3A4
Sf9 control
H2O control

G


LucPEE
RLU
15000
10000
5000
0
1A1
1A2
2A6
2B6
2C8
2C9
2C19
2D6
2E1
3A4
Sf9 control

H


LucGE
RLU
400000
300000
200000
100000
0
1A1
1A2
2A6
2B6
2C8
2C9
2C19
2D6
2E1
3A4
Sf9 control
H2O control

I

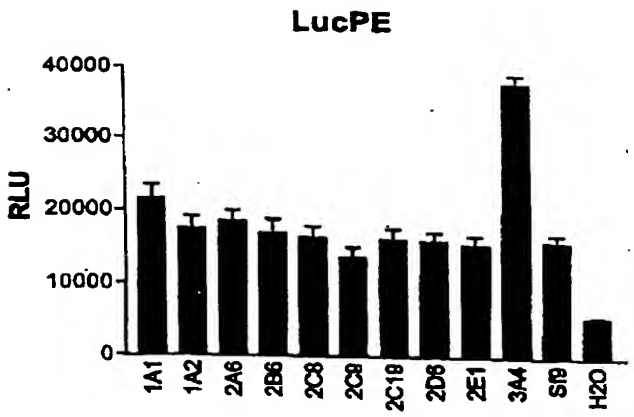

LucPE
RLU
40000
30000
20000
10000
0
1A1
1A2
2A6
2B6
2C8
2C9
2C19
2D6
2E1
3A4
S19
H2O

Figure 4

Time-dependence of cyp450/substrate incubation in two-step luminescent cyp450 reactions using D-luciferin derivatives.

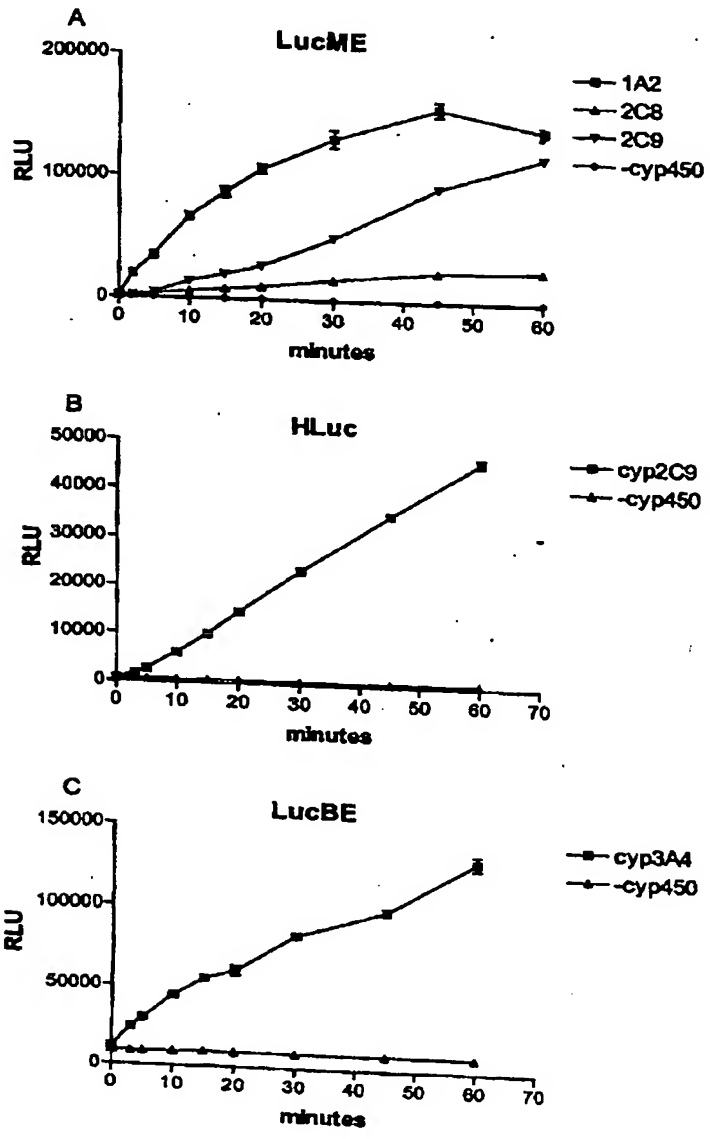


Figure 5

Time course of light output from two-step luminescent cyp450 reactions using LucME.

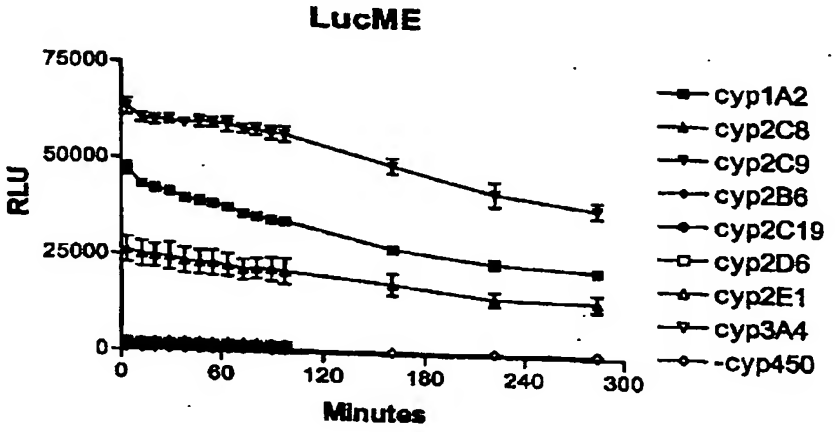


Figure 6

One-step luminescent cyp450 assays at room temperature using LucME.

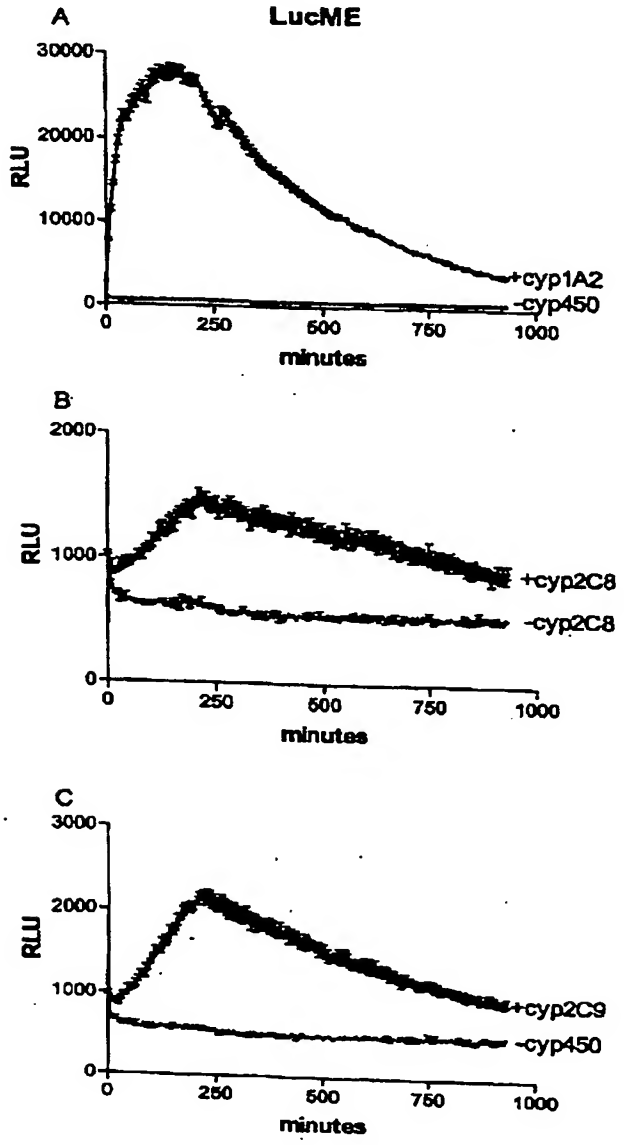


Figure 7

One-step luminescent cyp450 assays at 37°C using D-luciferin derivatives.

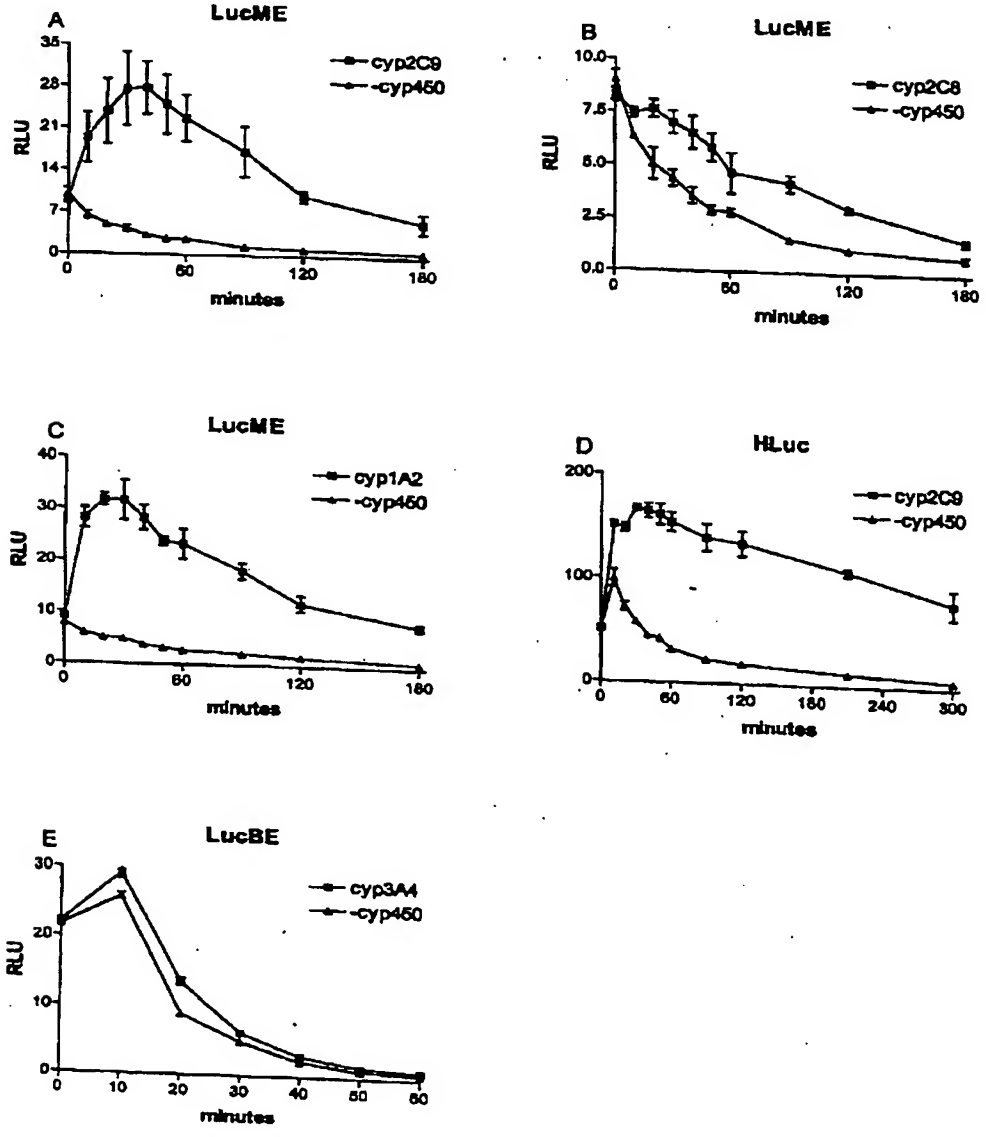


Figure 8

Pooled human liver microsomes in two-step luminescent cyp450 reactions using D-luciferin derivatives.

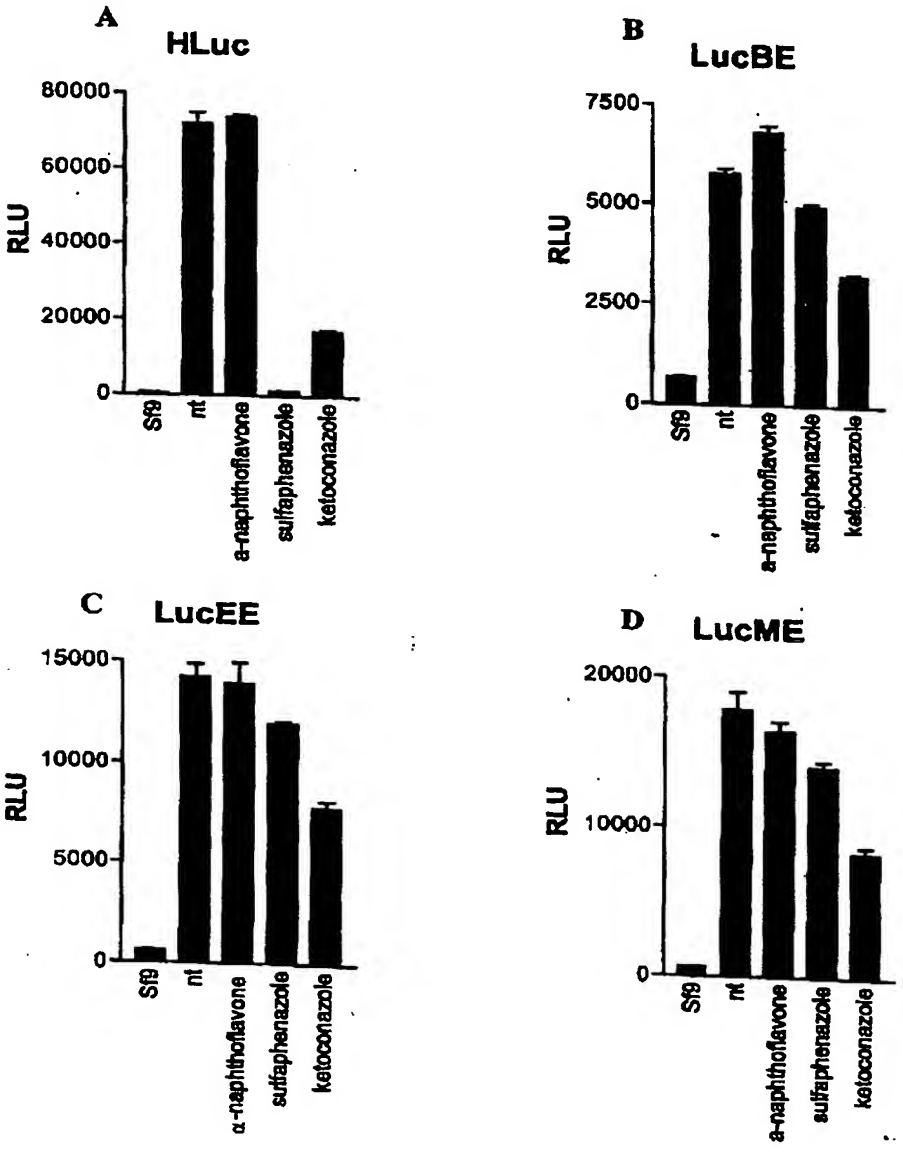


Figure 9

Two-step detection of de-picolinylase activity picolinyl D-luciferin derivatives

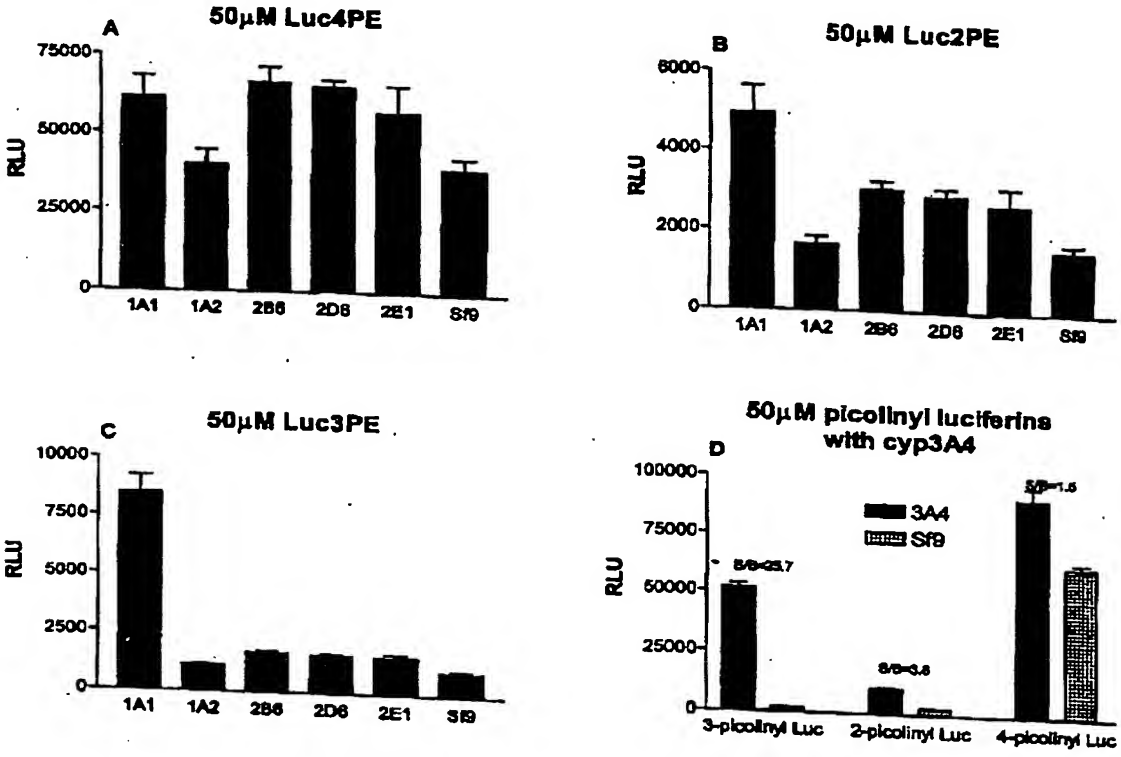
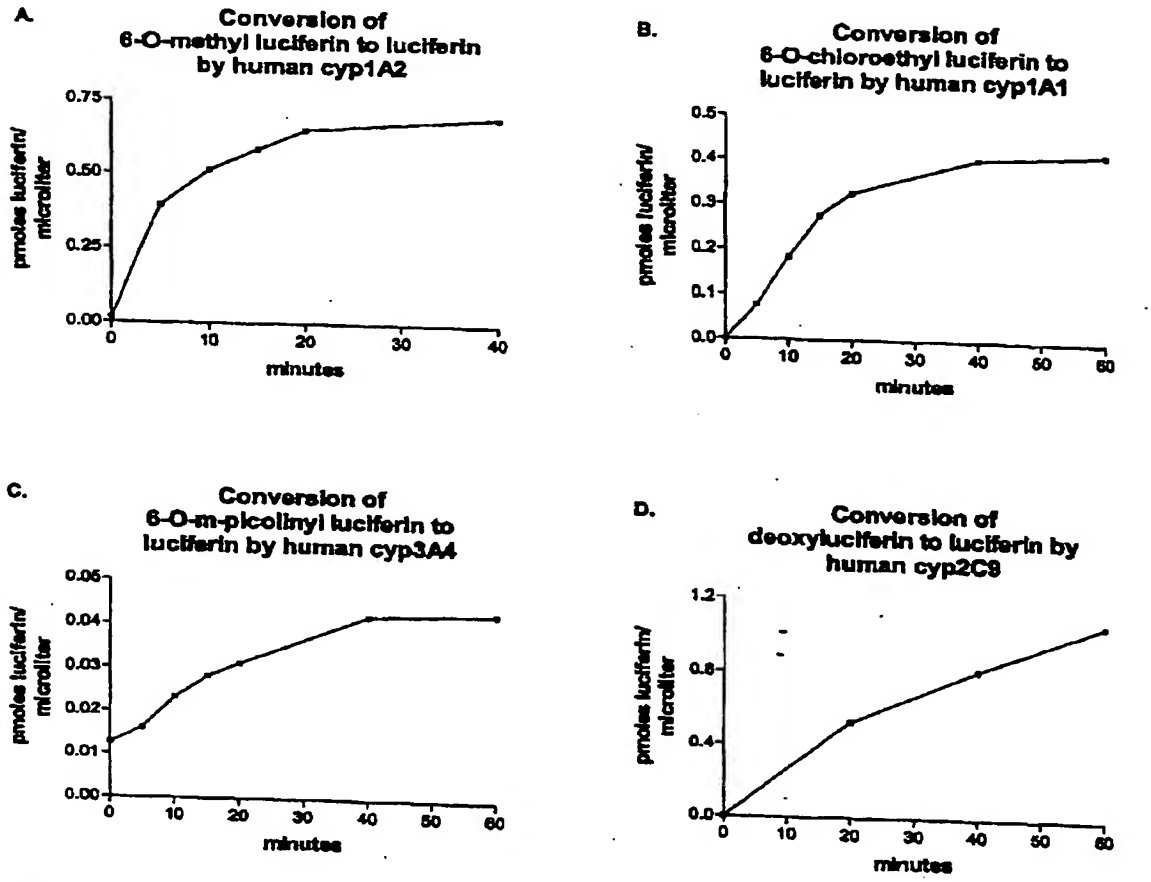


Figure 10

Conversion of D-luciferin derivatives to luciferin by human cyp450s



100 micromolar 6-O-methyl luciferin (panel A.), 100 micromolar 6-O-chloroethyl luciferin (panel B.), 25 micromolar 6-O-m-picolinyl luciferin (panel C.) or 100 micromolar deoxyluciferin (panel D.), was incubated with cyp1A2, cyp1A1, cyp3A4 or cyp2C9, respectively, in reaction volumes of 150 microliters at 37°C. At the indicated times reactions were stopped by addition of tergitol to 0.1% (v/v) and flash freezing in liquid nitrogen. 95 microliters of each reaction was subjected to fractionation by HPLC and luciferin was detected by fluorescence. The zero time points were determined by inactivating the cyp450 with 0.1% tergitol prior to substrate addition (cyp1A1, 1A2 and 3A4) or by determining the luciferin content of a no enzyme control with deoxyluciferin (cyp2C9).

HPLC method: High-pressure liquid chromatography was performed on an HP 1050 LC system equipped with a multi-wavelength absorbance (HP 1050 MWD) and fluorescence detector (HP 1046A). Separation was achieved on a 5 micron Adsorbosphere HS C18 column (Alltech Associates) with a solvent gradient of 0.05M KH_2PO_4 / pH 6 (solvent A) and 80:20 acetonitrile/water (solvent B). The gradient conditions used were 15%B to 95%B over 10 min. Substrates were detected by absorbance at either 262 or 330 nm and Luciferin was detected by fluorescence at 520 nm (emission) with an excitation wavelength of 330 nm.

Figure 11

Inhibition of cyp450 by known cyp450 substrates.

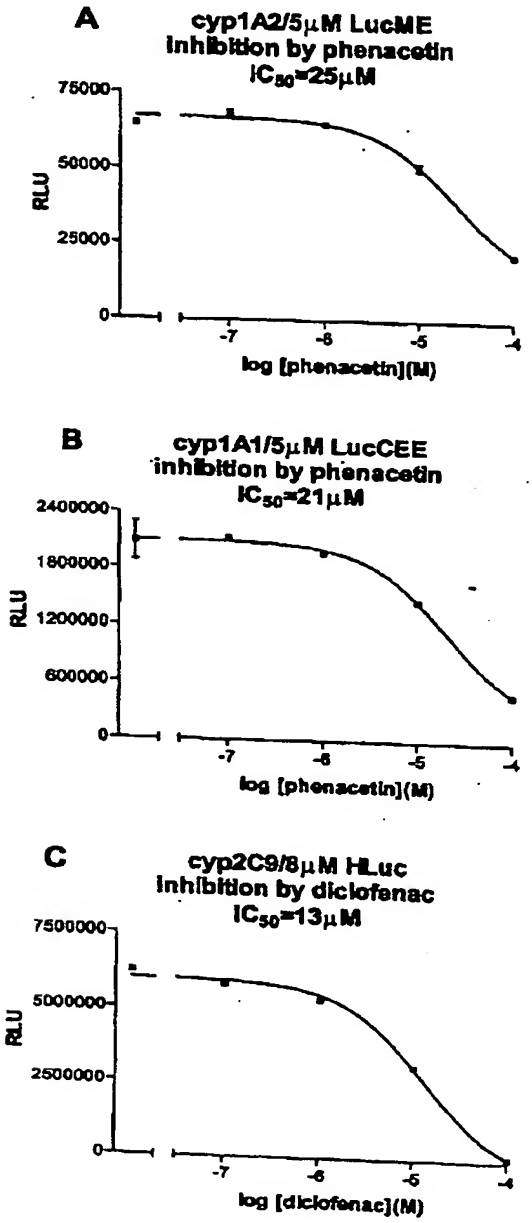


Figure 12

P450 action on methoxy-coelenterazine HH, coelenterazine HH, and coelenterazine by chemiluminescent and bioluminescent detection.

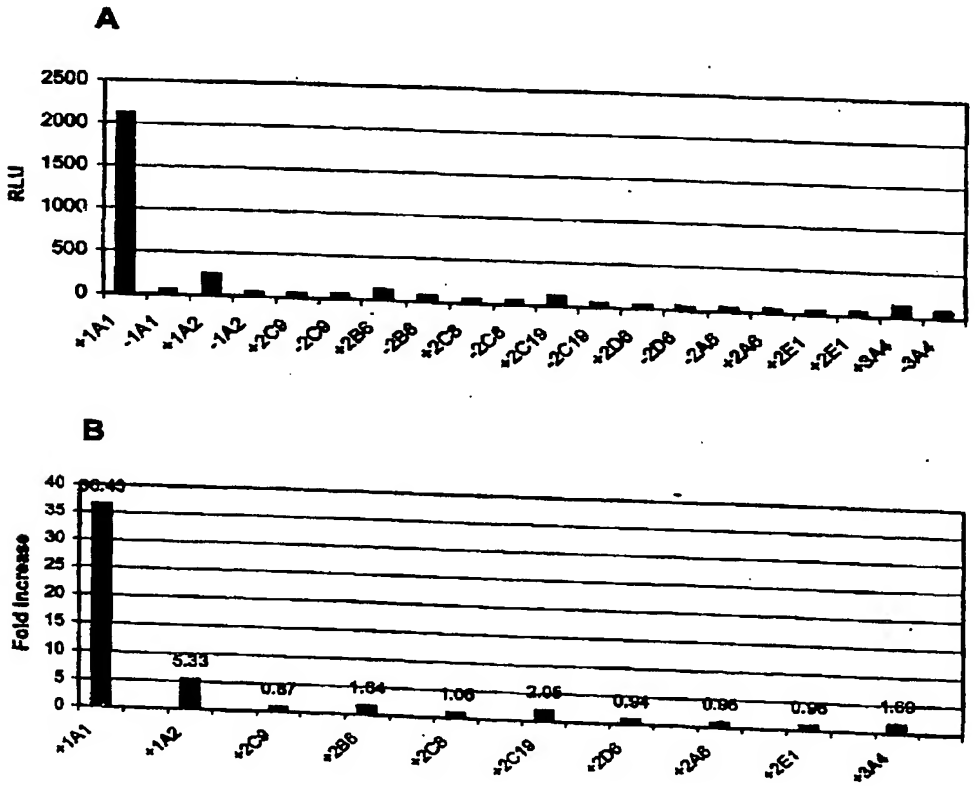
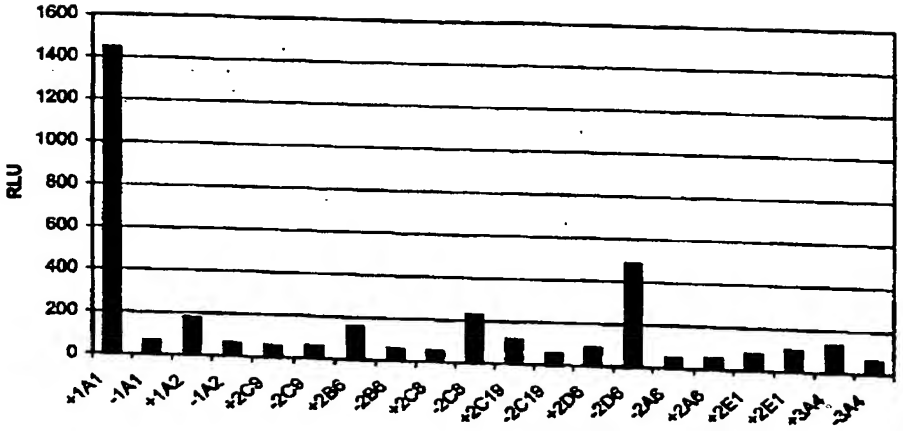


Figure 12 cont.

C



D

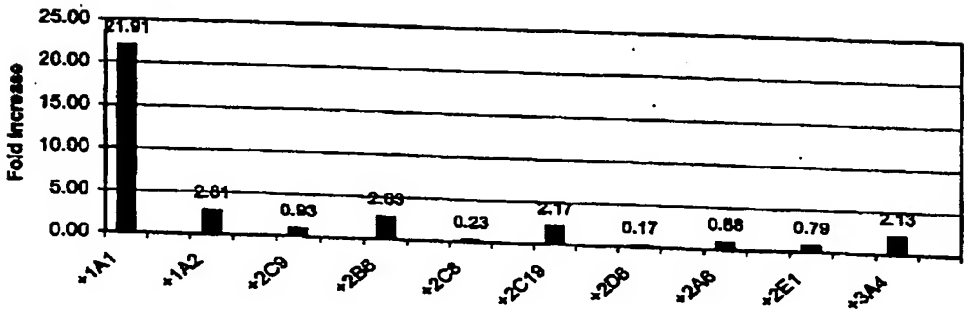
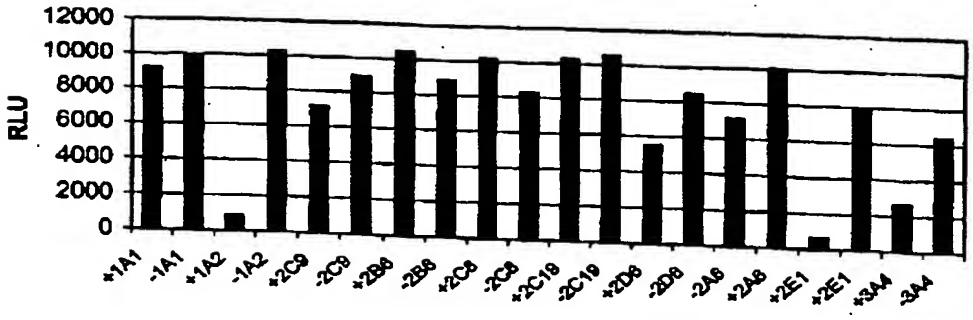


Figure 12 cont.

E



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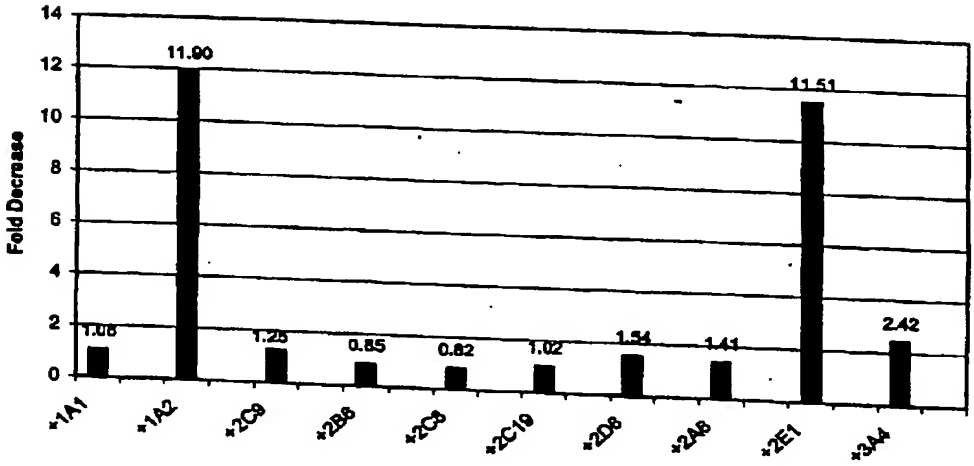
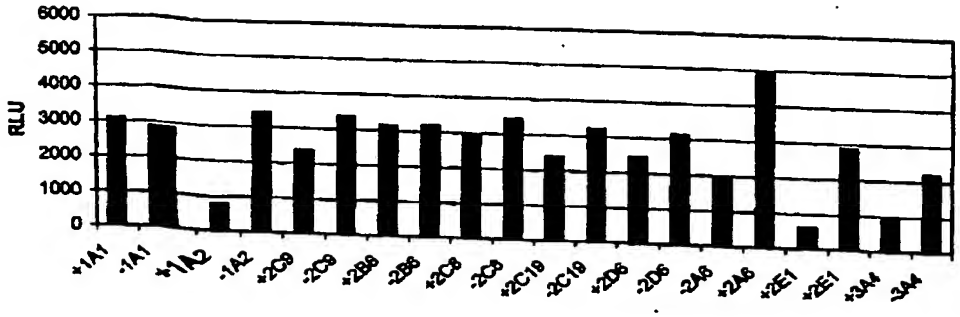


Figure 12 cont.

G



H

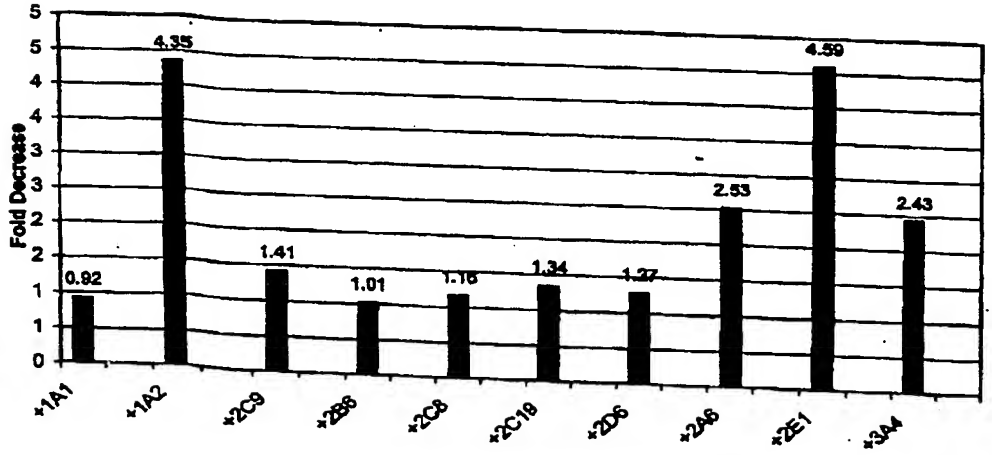


Figure 12 cont.

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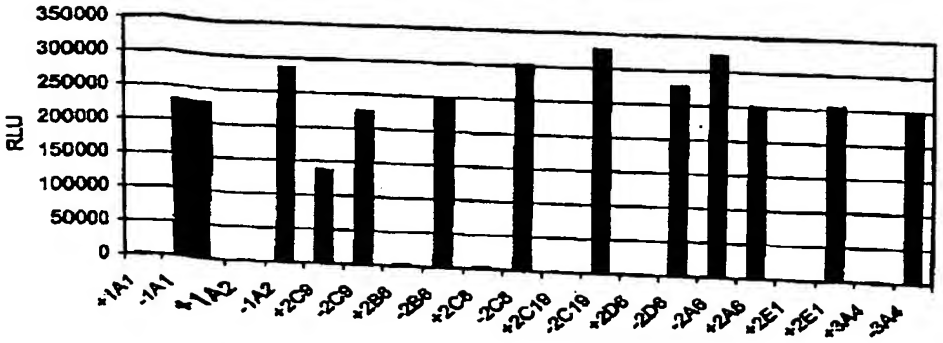

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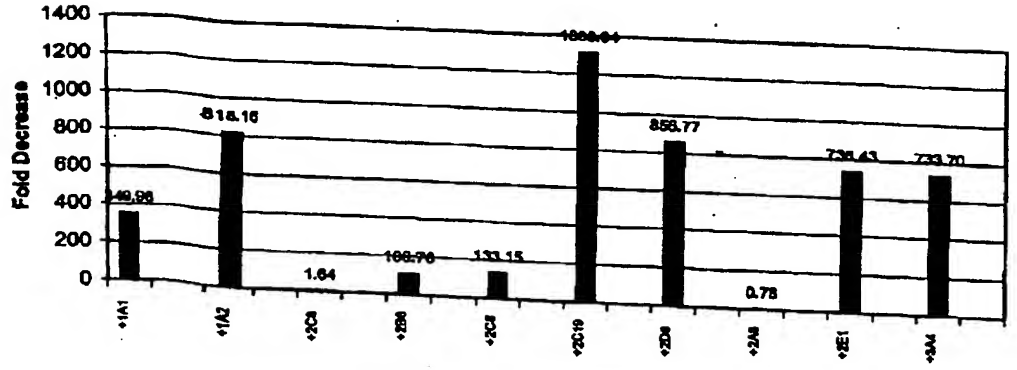


Figure 12 cont.

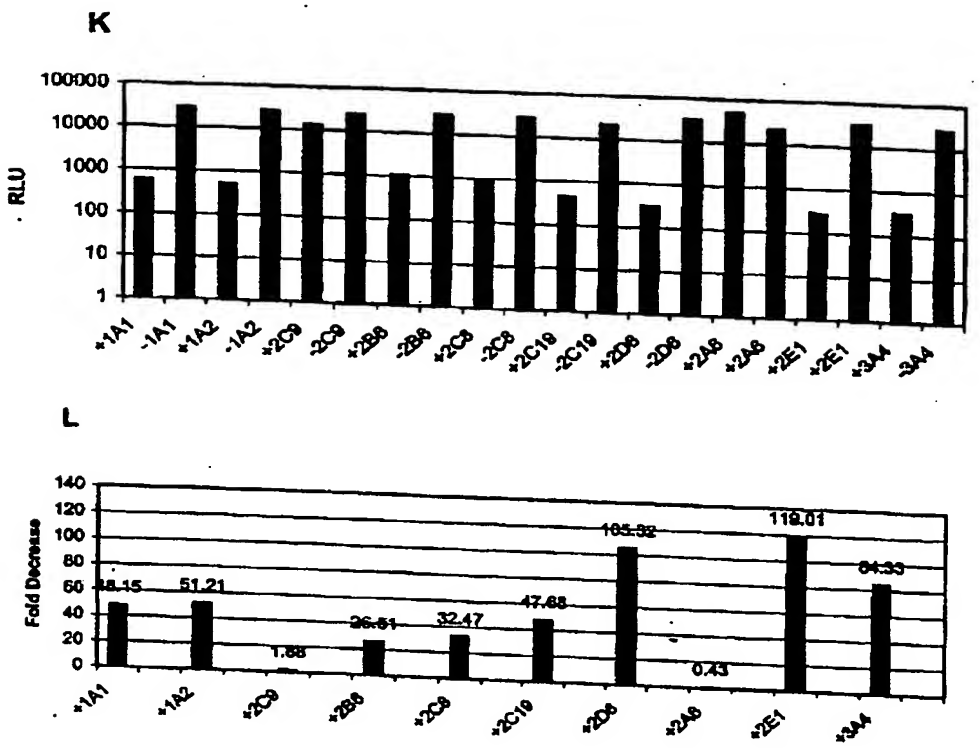


Fig. 13: Pyrophosphatase Activity

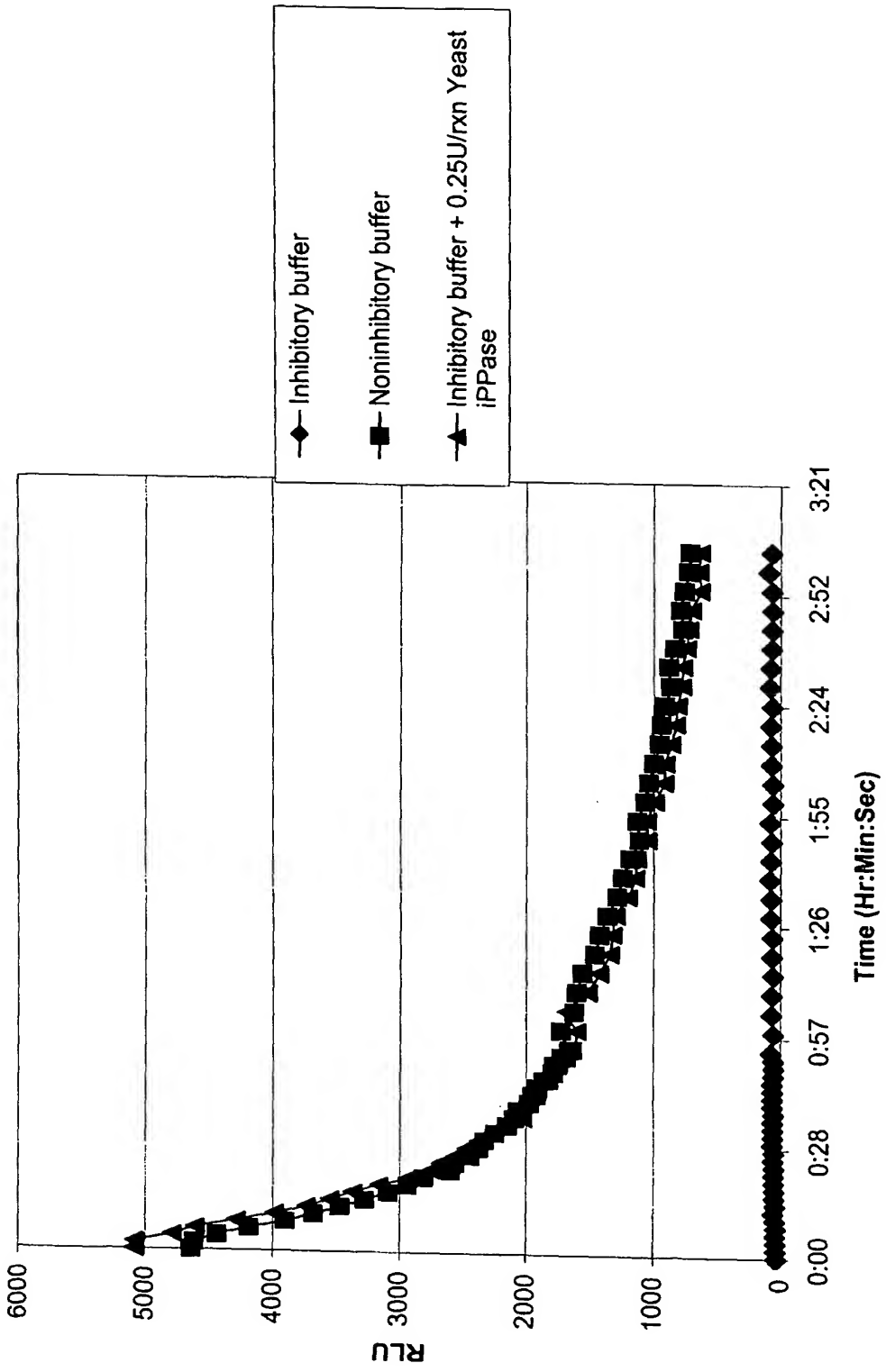


Figure 14

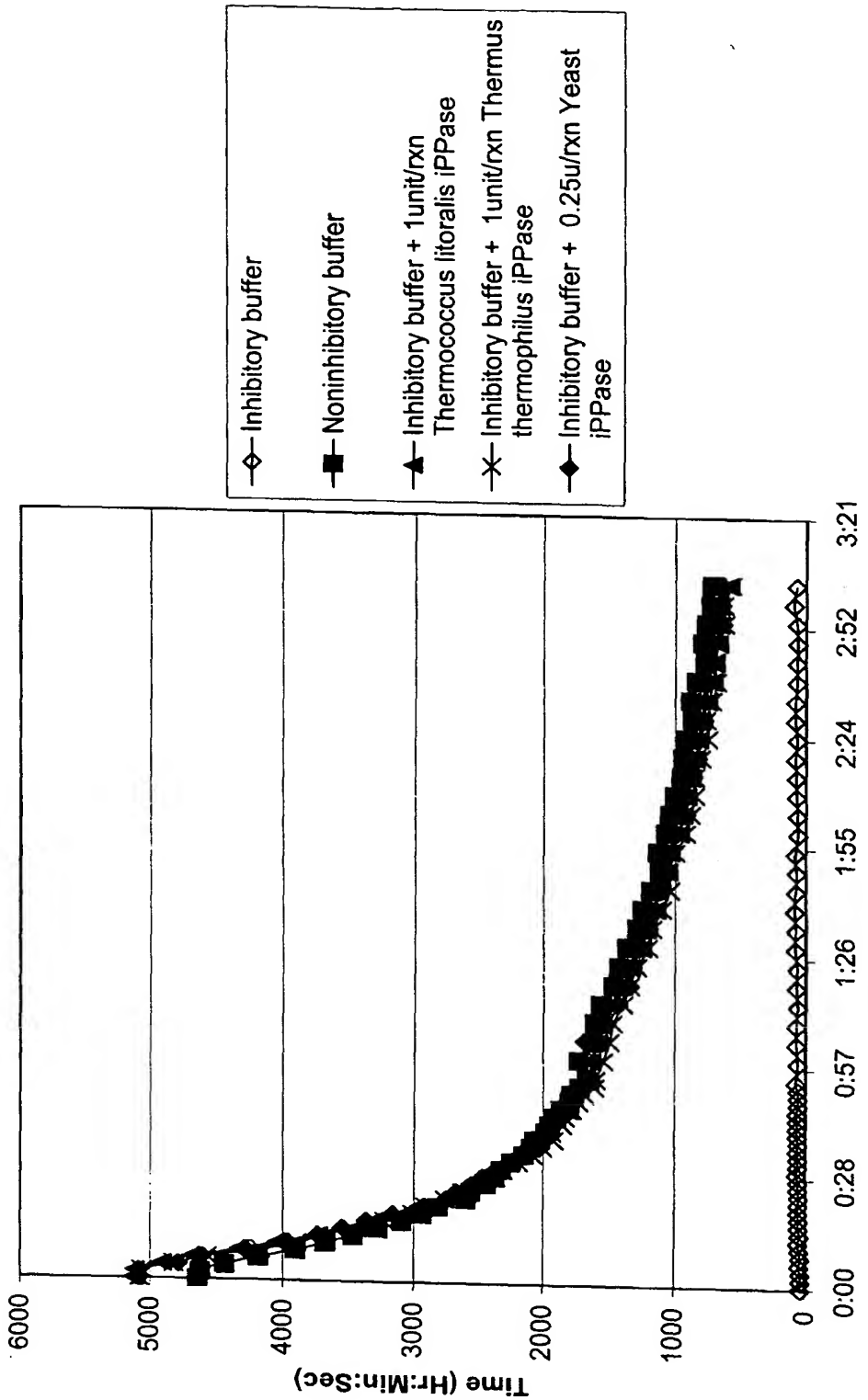

Inhibitory buffer
Noninhibitory buffer
Inhibitory buffer + 1unit/rxn Thermococcus litoralis iPPase
Inhibitory buffer + 1unit/rxn Thermus thermophilus iPPase
Inhibitory buffer + 0.25u/rxn Yeast iPPase
RLU
Time (Hr:Min:Sec)
0
1000
2000
3000
4000
5000
6000
0:00
0:28
0:57
1:26
1:55
2:24
2:52
3:21

Figure 15

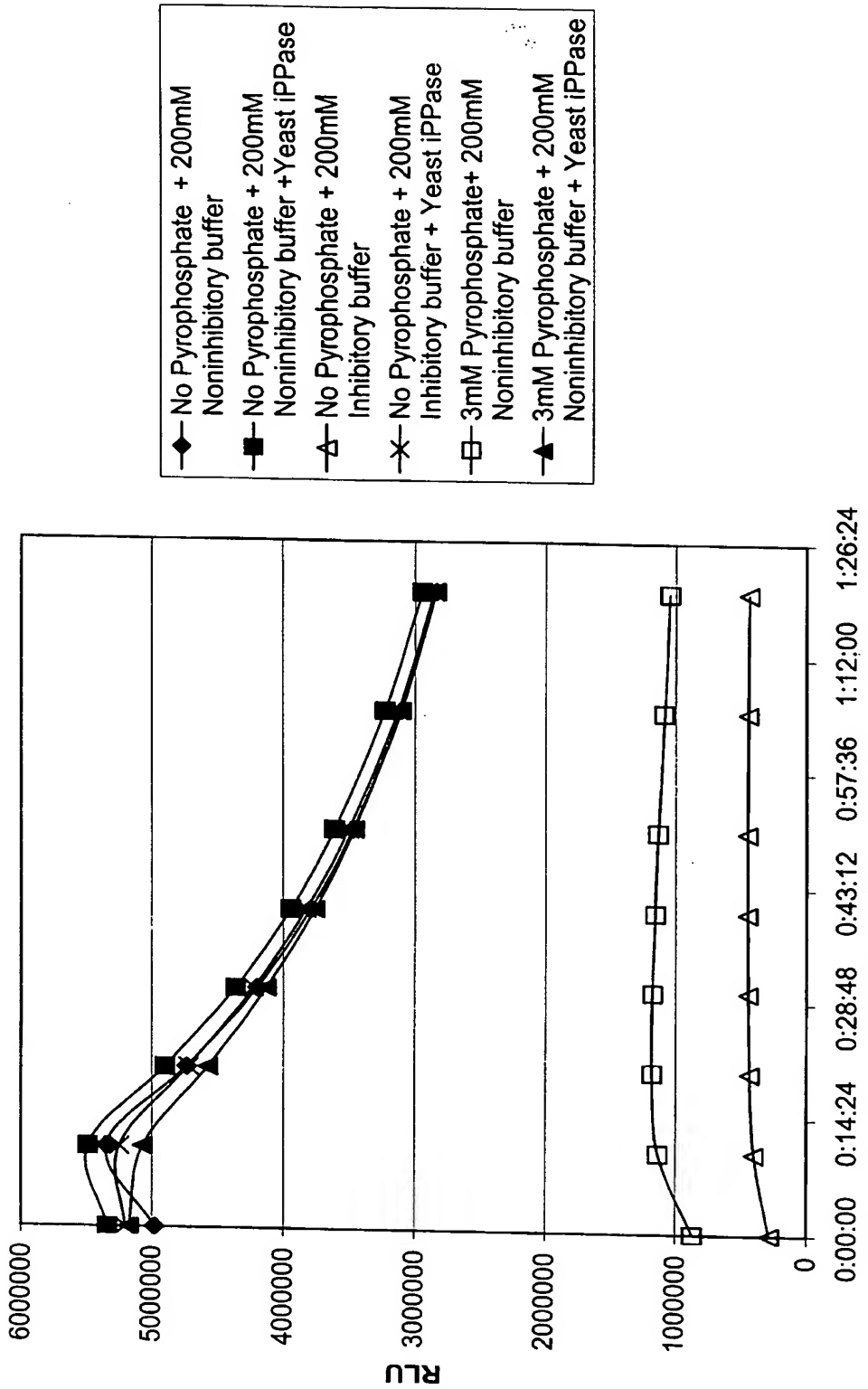

No Pyrophosphate + 200mM Noninhibitory buffer
No Pyrophosphate + 200mM Noninhibitory buffer +Yeast iPPase
No Pyrophosphate + 200mM Inhibitory buffer
No Pyrophosphate + 200mM Inhibitory buffer + Yeast iPPase
3mM Pyrophosphate+ 200mM Noninhibitory buffer
3mM Pyrophosphate + 200mM Noninhibitory buffer + Yeast iPPase
RLU
6000000
5000000
4000000
3000000
2000000
1000000
0
0:00:00
0:14:24
0:28:48
0:43:12
0:57:36
1:12:00
1:26:24
Time (Hr:Min:Sec)

Figure 16

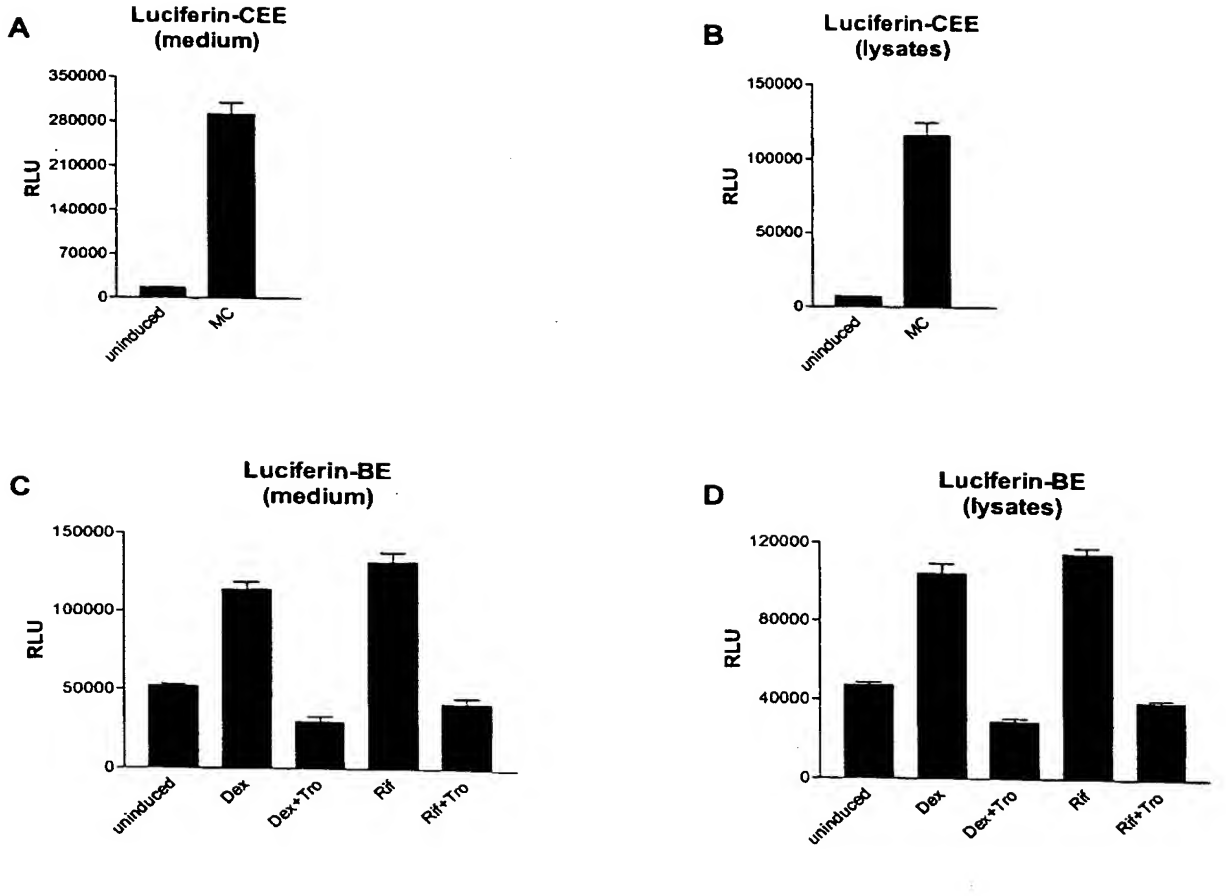
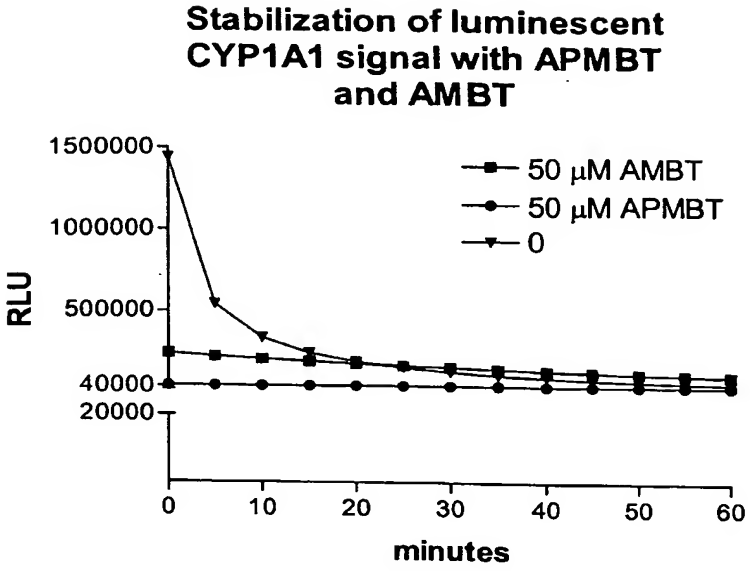

A
Luciferin-CEE
(medium)
RLU
350000
280000
210000
140000
70000
0
uninduced
MC
B
Luciferin-CEE
(lysates)
RLU
150000
100000
50000
0
uninduced
MC
C
Luciferin-BE
(medium)
RLU
150000
100000
50000
0
uninduced
Dex
Dex+Tro
Rif
Rif+Tro
D
Luciferin-BE
(lysates)
RLU
120000
80000
40000
0
uninduced
Dex
Dex+Tro
Rif
Rif+Tro

FIGURE 17


Stabilization of luminescent CYP1A1 signal with APMBT and AMBT
1500000
1000000
500000
40000
20000
RLU
50 µM AMBT
50 µM APMBT
0
0
10
20
30
40
50
60
minutes